STANDARD OPERATING PROCEDURE

Training & Education

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1. Policies

1.1. All users of the cytometers in the Flow Cytometry and Cell Sorting Facility must receive training in principles and instrumentation, instrument operations, quality control (QC), reagents and panel design, acquisition and measurements, analysis and statistics prior to beginning work in this shared resource laboratory (SRL). Users who wish to conduct experiments outside of normal operating hours (Monday through Fridays, 9AM-5PM) must receive advanced training to ensure independence.

1.2. Ancillary equipment users (Vi-Cell XR, Curiox HT2000, OctoDissociator) must be trained in proper operation of equipment.

2. Purpose

2.1. To ensure competency in both the theoretical and technical aspects of flow cytometry as the first step fo promote rigor and reproducibility in flow cytometry experiments

2.2. Prevent misuse of equipment

2.3. To learn best practices in the field of flow cytometry

3. Responsibilities

3.1. Users may not schedule any of the cytometers without first receiving equipment specific training.

3.2. The FCCSF Manager and FCCSF Director set all training requirements which are reviewed and endorsed by the FCCSF Advisory Board.

4. Procedures

4.1. **Onboarding**

4.1.1. Users contact facility manager, Robbie Moore, either by email or in person and specify which equipment on which they wish to receive training.

4.1.2. Facility Manager emails Onboarding Document to customer. The Onboarding document contains the following information:

4.1.2.1. How to sign up for an iLab account and access scheduling calendars

4.1.2.2. Rates for all instruments

4.1.2.3. Policies

4.1.2.4. Information on capabilities of each instrument in the facility

4.1.2.5. Links to training documents and videos

4.2. **BD Fortessa X-20 Training**

4.2.1. Sign up for an account at https://fluorofinder.com/ using your @tamu.edu email. This will enable you to access all the premium features of FluoroFinder including access to video training courses.

4.2.2. Log in to FluoroFinder and navigate to Flow Cytometry Training. Complete the Flow Cytometry Essentials before coming to your first hands-on training.

4.2.3. Review Hands-On training document Setting up an experiment in Diva on the Fortessa. Review video BD Fortessa X-20 Setting Up an Experiment in Diva Software

4.2.4. Schedule a consultation with facility manager to discuss the specifics of your experiment and get recommendations on which instrument to use, sample processing and assay specific best practices.

4.2.5. Schedule an in-person, hands-on, equipment-specific training on the Fortessa in iLab (based on staff availability.) This training takes about one hour and covers the following topics:

4.2.5.1. Fortessa lasers and filter sets (configuration)

4.2.5.2. Setting up an experiment in FACSDiva Software

4.2.5.2.1. Adding and naming specimens and tubes

4.2.5.2.2. Setting up workspace – create appropriate plots, tables and gates

4.2.5.2.3. Deleting unwanted parameters

4.2.5.2.4. Using Experiment Layout to label fluorophores, add keywords and setting stopping criteria

4.2.5.3. Adjusting voltages and thresholds

4.2.5.4. Running compensation

4.2.5.5. Instrument Operation and monitoring

4.2.5.5.1. Loading and removing a tube from the SIT

4.2.5.5.2. Flow rate adjustment optimization

4.2.5.5.3. Monitor fluidics and signal stability with a time plot

4.2.5.6. Data acquisition and recording

4.2.5.7. Data export

4.2.5.8. Cleaning the instrument after use

4.2.5.9. After completing training and demonstrating proficiency at the above listed tasks, users may then schedule time on and use the Fortessa. For their

first run, the FCCSF Manager is on-site to assist, answer questions and verify that the user is competent to use the instrument independently moving forward. Users not yet proficient may schedule assisted use.

4.3. **BD Fortessa X-20 After Hours and Weekend Training** Advanced users who require time to run experiments outside normal operating hours (9AM-5PM Monday-Friday) must complete the After Hours and Weekend Training

4.3.1. Contact facility manager to schedule

4.3.2. This training takes approximately 15 minutes and is based on the After-Hours training document. Topics covered include:

4.3.2.1. Starting up and shutting down the instrument

4.3.2.2. Check state of fluidics cart, change sheath boxes and empty waste

4.3.2.3. Running and assessing Cytometer Setup and Tracking (CS&T)

4.3.2.4. Troubleshooting tips and link to Fortessa Troubleshooting YouTube Video

4.4. **BD Fortessa X-20 HTS Training**

4.4.1. Complete Fortessa general training

4.4.2. Review FCCSF training document BD Fortessa X-20 HTS

4.4.3. Review FCCSF YouTube Video Fortessa Plate-Mode Protocol Tutorial

4.4.4. Review FCCSF YouTube Video BD Fortessa X-20 - Setting up in Diva Software for HTS mode

4.5. **Cytek Aurora 5L Training**

4.5.1. Sign up for an account at https://fluorofinder.com/ using your @tamu.edu email. This will enable you to access all the premium features of FluoroFinder including access to video training courses.

4.5.2. Log in to FluoroFinder and navigate to Flow Cytometry Training FluoroFinder.com/flow_cytometry_training. Complete the following video training courses;

4.5.2.1. Flow Cytometry Essentials

4.5.2.2. Spectral Flow

4.5.2.3. Review Cytek SpectroFlo Tutorials

4.5.2.3.1. Create and Unmix and Experiment

4.5.2.3.2. SpectroFlow Overview

4.5.3. Spectral Best Practices Training – Because of the number of parameters available and the sensitivity of the instrument, it is absolutely crucial to follow best practice in panel design and reference control optimization.

4.5.3.1. Review the following videos:

4.5.3.1.1. Applying Panel Design Principles

4.5.3.1.2. Best Practices for Reference Controls Optimization

4.5.3.1.3. SpectroLearn Panel Design Best Practices

4.5.3.2. Consult with facility staff and/or Cytek application to plan panel design, QC reference controls, QC unmixing and autofluorescence extraction options as needed.

4.5.4. Cytek Aurora 5-L Hands on Training

4.5.4.1. Contact facility manager to schedule in-person, hands-on training. This training takes about one hour, and covers the following topics:

4.5.4.1.1. Cytek lasers and filter arrays

4.5.4.1.2. Setting up an experiment in SpectroFlow including the following:

4.5.4.1.2.1. Create and open an experiment

4.5.4.1.2.2. Choose fluorescent tags

4.5.4.1.2.3. Create groups, tubes or well plate layout

4.5.4.1.2.4. Labeling fluorophores with marker names

4.5.4.1.2.5. Setting stopping criteria

4.5.4.1.2.6. Loader settings

4.5.4.1.2.7. Add keywords

4.5.4.1.2.8. Worksheet elements on both raw and unmixed worksheets

4.5.4.1.3. Adjusting FSC, SSC and SSC-B voltages and adjusting FSC threshold

4.5.4.1.4. Data acquisition and recording

4.5.4.1.5. Monitor fluidics and signal stability with a time plot

4.5.4.1.6. Unmix data

4.5.4.1.6.1. Running reference controls,

4.5.4.1.6.2. checking spectral signatures of reference controls,

4.5.4.1.6.3. unmixing

4.5.4.1.6.4. checking quality of unmixing.

4.5.4.1.6.5. Troubleshoot unmixing.

4.5.4.1.7. Exporting data and managing data on instrument

4.5.4.1.8. Running cleaning after use protocol after use

4.5.4.1.9. End of day shut down and power off instrument

4.5.4.1.10. Once theoretical and hands-on training are complete and users demonstrate proficiency at the above listed tasks, users are then able to schedule time on the Cytek Aurora 5-L and independently run experiments

during normal operating hours (9AM-5PM Monday-Friday). Users not yet proficient after one training session may schedule assisted use.

4.6. **Cytek Aurora 5-L After Hours training** Advanced users who require time to run experiments outside normal operating hours (9AM-5PM Monday-Friday and weekends) must complete the After Hours and Weekend training. This training takes about 15 minutes and includes the following topics. 4.6.1. Starting up and shutting down the instrument

4.6.2. Checking waste and sheath levels.

4.6.3. Refilling sheath and emptying waste.

4.6.4. Running QC

4.6.5. Review of Troubleshooting section of Cytek Aurora User's Guide

4.7. **BDFACS Aria III training** Sorting on the Aria is a high skill level, so sorts will be set up and run by facility staff in most cases. Users should have basic flow cytometry knowledge, a basic theory of droplet-based sorting, how to select nozzle size and sample prep for sorting.

4.7.1. Complete online video training

4.7.1.1. Flow Cytometry Essentials course if not already completed

4.7.1.2. FluoroFinder Sorting

4.7.2. Consult with facility staff

4.7.2.1. Review Reference Guide for Cell Sorting Sample Preparation and decide on nozzle size, event rate, sample and collection vessel type, sort temperature, sort buffer and collection media/buffer that are best for your sample type.

4.7.2.2. BDFACS Aria Configuration Review configuration

4.7.2.3. Schedule sort in iLab based on FCCSF staff availability

4.8. **BD FACSAria III self-user designation** –

4.8.1. Most sorts are run by FCCSF staff. However, a user can be designated as a self-user and sort for lower rate after they meet the following criteria;

4.8.1.1. Have observed at least three sorts

4.8.1.2. Have been trained by facility staff and can demonstrate proficiency in; identifying problems with the stream (fanning, instability), stopping the stream, sonicating the nozzle, monitoring fluidics and signal stability and restarting both the stream and the sort process.

4.8.1.3. Have been trained by facility staff and can demonstrate proficiency in cleaning after use and shutdown of the BDFACS Aria III.

4.9. **Miltenyi MACSQuant Tyto training**

4.9.1. Complete Flow Cytometry Essentials course if not already completed

4.9.2. Review Tyto Training Document

4.9.3. Consult with facility staff to discuss sample prep and necessary supplies.

4.9.4. Run first sort with facility staff assistance. User will be designated trained by facility staff once they deem that the user is proficient in the following areas:

4.9.4.1. Priming and loading cartridge

4.9.4.2. Setting up workspace

4.9.4.3. Monitoring sort

4.9.4.4. Familiarity with troubleshooting section of the Tyto manual

4.9.4.5. After completing training and demonstrating proficiency at the above listed tasks, users may then schedule time on and use the MACSQuant Tyto Cell Sorter. For their first run, the FCCSF Manager is on-site to assist, answer questions and verify that the user is competent to use the instrument independently moving forward. Users not yet proficient may schedule assisted use.

4.9.4.6. Once proficiency is achieved, Users do not need any additional training to use the Tyto after hours or on weekends due to its ease of use.

4.10. **Vicell XR Cell Viability Analyzer Training** - This is a benchtop instrument that automates the trypan blue dye exclusion method of assessing cell count and viability. Training consists of the following;

4.10.1. Watch FCCSF YouTube video Vi-Cell XR Protocol Tutorial This training takes approximately 5 minutes to finish, Upon completion, users are now allowed to use the Vi-Cell as needed.

4.11. **Miltenyi gentleMACS OctoDissociator Training** - consists of the following;

4.11.1. Watch FCCSF YouTube video gentleMACS octoDissociator Protocol Tutorial covering the following topics: loading and removing C-tubes and heaters, selecting the appropriate program and cleaning any spills.
NOTE: Users are required to purchase C-tubes and dissociation kits directly from Miltenyi prior to using this instrument.

4.11.2. This video takes approximately 2.5 minutes to finish. Upon completion, users can schedule time on the OctoDissociator.

4.12. **Agilent Seahorse XFe96 Training**

4.12.1. Watch Agilent Seahorse XF Training Webinar Prepare for your XF Assay, Gather Assay Materials and Set Up to Run XF Assays

4.12.2. Contact Seahorse Application Scientist jay.dunn@agilent.com for specific questions regarding which kit to use.

4.12.3. Upon completion, users can schedule time on the Seahorse

4.13. **Curiox HT2000 Laminar Flow Cell Washer Training –** this is a hands-on, in-person training and includes the following;

4.13.1. Purchase of proprietary consumables needed; a cap for washing buffer and specialized Curiox plates

4.13.2. Contacting Curiox Application Scientist for recommendations on a wash protocol for your specific sample type.

4.13.3. Instrument use including startup, calibrating, wash settings, and shut down.

4.13.4. This training takes approximately 45 minutes to finish. With successful completion of the in-person training, users are allowed to schedule and use the Curiox as needed.

5. Date established and subsequent reviews (annually at a minimum)

5.1. List the date of establishment

5.2. List dates of subsequent reviews or changes to the procedure.

Revision	Review or Revision Date	Description of Changes	Reviewed By
0.0	11/01/24		RM and EVS